

skilled in the art. To minimize any undesired binding on the surfaces of the electrodes **46** and **48**, a blocking agent may also be applied such as described above.

**[0033]** The leads for the electrodes may generally be positioned in any desired manner as is readily appreciated by those skilled in the art. Referring to **FIGS. 1-2**, for instance, the leads **43** and **45** for the electrodes **42** and **44**, respectively, are positioned on the first substrate **80**, while the leads **47** and **49** for the electrodes **46** and **48**, respectively, are positioned on the second substrate **40**. In the illustrated embodiment, the leads **43**, **45**, **47**, and **49** are positioned parallel to the flow of the test sample. Alternatively, the leads **43**, **45**, **47**, and/or **49** may be positioned perpendicular to the flow of the test sample. For example, **FIG. 5** illustrates an embodiment in which the leads **43**, **45**, **47** and **49** are positioned perpendicular to the flow of the test sample.

**[0034]** A variety of other components may also be employed on the first and/or second substrates **40** and **80**. For example, in one embodiment, several flow-control mechanisms are used in conjunction with the first substrate **80**, although it should be understood that such mechanisms may also be used in conjunction with the first substrate **40**. For example, an absorbent wicking pad **28** is disposed at one end of the substrate **80** to promote capillary action and fluid flow of the sample. To ensure that the absorbent wicking pad **28** does not inhibit the ability of the electrodes from being placed sufficiently close to each other upon formation of the assay device, the first substrate **40** may define a cut-out region that corresponds to the size and shape of the absorbent wicking pad **28**. Thus, when the substrates **40** and **80** are placed in a face-to-face relationship, the absorbent wicking pad **28** fits into the cut-out region.

**[0035]** In addition, a sample channel **14** is also formed on the substrate **80**. Multiple sample channels **14** may be utilized for multiple test samples. The sample channel **14** may be formed from any of a variety of materials through which the test sample is capable of flowing. In most embodiments, it is desired that a dielectric material be used to form the sample channel **14** to reduce unwanted interference with the electrochemical detection of the analyte. The term "dielectric" material generally refers to a material having a dielectric constant "k" of less than about **5** at **1 kHz** (defined by ASTM D150-98 Standard Test Methods for AC Loss Characteristics and Permittivity (Dielectric Constant) of Solid Electrical Insulation, an insulation resistance of greater than **10 GΩ/mil**, and/or a breakdown voltage of greater than **1000 V/mil DC** (also defined by ASTM D150-98 Standard Test Methods for AC Loss Characteristics and Permittivity (Dielectric Constant) of Solid Electrical Insulation. For example, a wide variety of organic and inorganic polymers, both natural and synthetic may be employed as a dielectric material for the sample channel **14**. Examples of such polymers include, but are not limited to, polyesters, polyimides, polyamides, polycarbonates, polyolefins (e.g., polyethylene, polypropylene, etc.), polysiloxanes, polyurethanes, polyvinylchlorides, polystyrenes, and so forth. Commercial dielectric materials, such as **5036 Heat Seal/Encapsulant**, **5018 UV curable dielectric**, **5018G UV curable dielectric** and **5018A UV curable dielectric** are available from DuPont Biosensor Group of Research Triangle Park, N.C.

**[0036]** If desired, such a polymeric channel may be formed by first applying monomer(s) or pre-polymer(s) for

the polymer, and then polymerizing the monomer(s) or pre-polymer(s) using well-known techniques, such as heating, irradiating, etc. For example, polymerization may be induced with ionizing radiation, which is radiation having an energy sufficient to either directly or indirectly produce ions in a medium. Some suitable examples of ionizing radiation that may be used in the present invention include, but are not limited to, ultraviolet radiation, electron beam radiation, natural and artificial radio isotopes (e.g.,  $\alpha$ ,  $\beta$ , and  $\gamma$  rays), x-rays, neutron beams, positively charged beams, laser beams, and so forth. Electron beam radiation, for instance, involves the production of accelerated electrons by an electron beam device. Electron beam devices are generally well known in the art. For instance, examples of suitable electron beam devices are described in U.S. Pat. No. **5,003,178** to Livesay; U.S. Pat. No. **5,962,995** to Avnerv; U.S. Pat. No. **6,407,492** to Avnerv et al., which are incorporated herein in their entirety by reference thereto for all purposes.

**[0037]** The geometry of the sample channel **14** may be selected so that capillary forces assist the flow of the test sample through the sample channel **14**. For example, the sample channel **14** may have a cross-sectional shape that is circular, square, rectangular, triangular, v-shaped, u-shaped, hexagonal, octagonal, irregular, and so forth. The sample channel **14** may also be continuous or discontinuous, and may also contain continuous or discontinuous sample mixing islands to promote sample mixing. Further, in some embodiments, the sample channel **14** may be a "microchannel", which is a channel that allows for fluid flow in the low Reynolds number region where fluid dynamics are dominated by viscous forces rather than inertial forces. The formula for Reynolds number is as follows:

$$Re = \rho \delta^2 / \eta \tau + \rho \mu \delta / \eta$$

**[0038]** wherein,  $\mu$  is the velocity vector,  $\rho$  is the fluid density,  $\eta$  is the viscosity of the fluid,  $\delta$  is the characteristic dimension of the channel (e.g., diameter, width, etc.), and  $\tau$  is the time scale over which the velocity changes (where  $\mu/\tau = \delta \mu / dt$ ). Fluid flow behavior at steady state ( $\tau \rightarrow \infty$ ) is characterized by the Reynolds number,  $Re = \rho \mu \delta / \eta$ . Due to their small size and slow velocity, microchannels often allow fluids to flow in the low Reynolds number regime ( $Re$  less than about **1**). In this regime, inertial effects, which cause turbulence and secondary flows, are negligible, and viscous effects dominate the dynamics so that flow is generally laminar. Thus, to maintain laminar flow, it is sometimes desired that the characteristic dimension of the channel range from about **0.5 micrometers** and about **500 micrometers**, in some embodiments from about **1 micrometer** to about **200 micrometers**, and in some embodiments, from about **5 micrometers** to about **10 micrometers**.

**[0039]** The height or depth of the sample channel **14** may also vary to accommodate different volumes of the test sample. The sample channel **14** may contain opposing walls that are raised a certain height above the surface **29** of the substrate **80**. For example, the walls may have a height of from about **0.1** to about **500 micrometers**, in some embodiments from about **0.5** to about **250 micrometers**, and in some embodiments, from about **1** to about **100 micrometers**. In some embodiments, the height of the sample channel **14** is the combination of the printed channel and an adhesive layer (e.g., glue, double-sided tape, etc.) used, for instance, to laminate a porous membrane over the printed channel. The thickness of the adhesive layer may vary, for instance, from